# Use of Toxicogenomics Data in Risk Assessment: Case Study for a Chemical in the Androgen-Mediated Male Reproductive Development Toxicity Pathway

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## Toxicogenomics (TG)

**Genomics is the study of all the genes of a cell, or tissue, at the DNA (genotype), mRNA (transcriptome), or protein (proteome) level (U.S. EPA, 2002; Interim policy on genomics. Science Policy Council, Washington, DC. http://www.epa.gov/osa/spc/genomics.htm.). Thus, toxicogenomics is defined as the study of gene expression (mRNA and/or protein products) after exposure to a toxic agent. Microarray analysis is the technique for studying the global expression of mRNAs in a tissue. Real-time reverse transcriptase-polymerase chain reaction [RT-PCR] is often used to verify the mRNA expression of specific genes.**

## Current Use of TG in EPA Risk Assessments

**In 2002, the U.S. EPA's Science Policy Council (SPC) developed the Interim Policy on Genomics. This policy states that genomics may be used in EPA risk assessments on a case-by-case basis in a weight-of-evidence approach (U.S. EPA, 2002). Currently there is no EPA guidance for how to incorporate toxicogenomics data into chemical assessments. The National Center for Environmental Assessment Colloquium entitled "Current Use and Future Needs of Genomics in Ecological and Human Health Risk Assessment" (http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=149984) identified the need to perform a case study integrating TG data into a chemical assessment as a first step toward defining methods and approaches.**

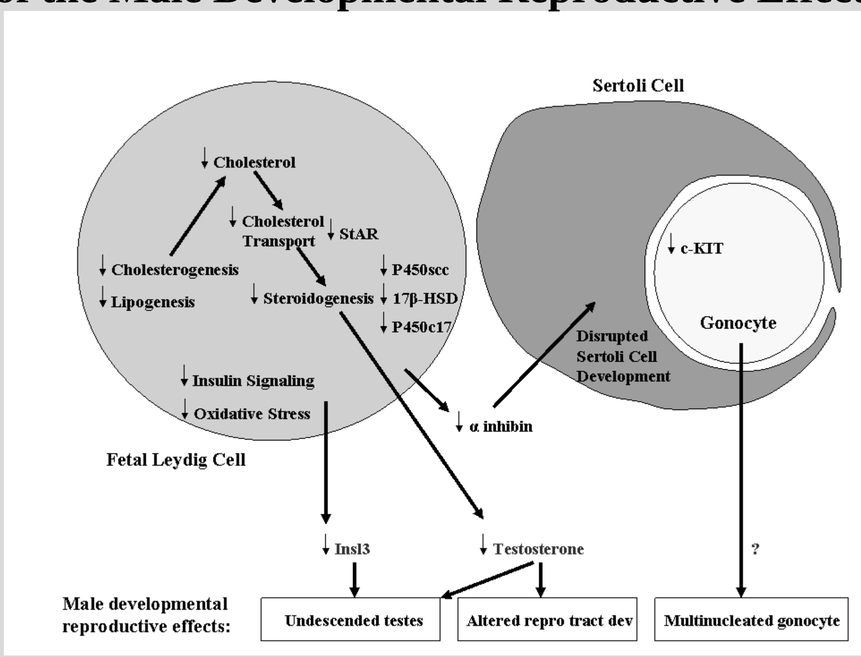
## Project Goals

- **Develop an approach for utilizing toxicogenomics data in a case study for a chemical with an ongoing or recent risk assessment**
- **In performing the case study:**
  - ✍**Identify risk assessment steps where toxicogenomics data may provide insight**
  - ✍**Develop a generic approach to integrating toxicogenomics data into risk assessments**

## Case Study Chemical Selection

**Dibutyl phthalate (DBP) was selected because it has a large and consistent TG dataset and an ongoing EPA assessment. The draft assessment includes some questions that the TG data may be able to address.**

### Proposed DBP Mechanism of Action that Explains Some of the Male Developmental Reproductive Effects

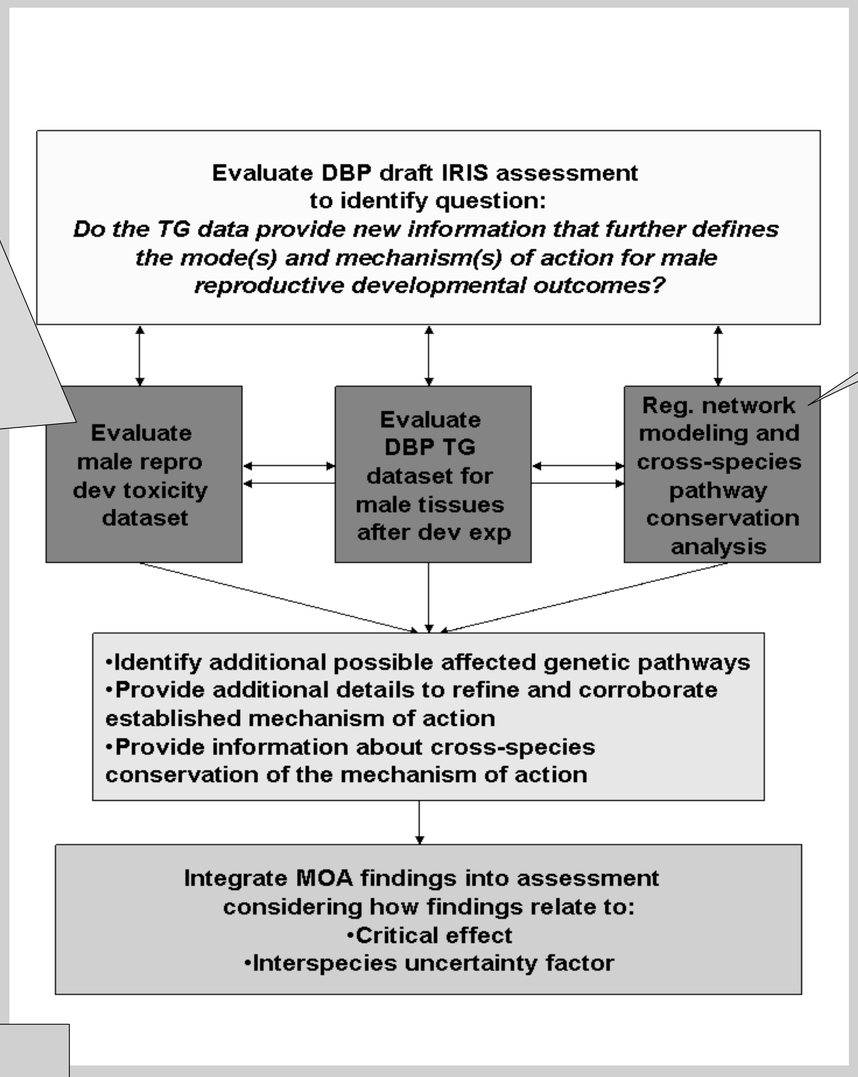



**The proposed mechanism of action for DBP is based on male reproductive developmental toxicity and toxicogenomics studies. Some of the affected pathways and individual genes whose expression has been shown to be affected by DBP exposure are included. The proposed mode of actions are shown in purple letters. Figure adapted from Liu et al. (2005), Shultz et al. (2001), Thompson et al. (2004), and Wilson et al. (2004).**

## Case Study Approach for Utilizing Toxicogenomics Information in Risk Assessment

**Male Reproductive Organ Effects from Selected Rat Toxicity Studies that Treated w/ 500 mg/kg/day DBP *in utero***

| Study | Effects |
|---|---|
| Barlow and Foster, 2003. Toxicol Pathol 31:397-410. | Fetal testes: Large aggregates of Leydig cells, multinucleated gonocytes, and increased no. of gonocytes; decreased no. of spermatocytes on PND 16 and 21<br>Seminiferous tubules: progressive mild (PND 45) to severe (PND 70) epithelial degeneration<br>Epididymes: decreased ductal coiling |
| Barlow et al., 2003. Toxicol Sci 73(2):431-41. | Fetal testes: Large aggregates of Leydig cells w/ lipid vacuoles |
| Bowman et al., 2005. Toxicol Sci 86:161-174. | Wolffian ducts: Marked underdevelopment characterized by decreased coiling |
| Carruthers and Foster, 2005. Birth Defects Research (Part B) 74:277-285. | Anogenital distance: reduction<br>Nipples and areolae: retained<br>Epididymes: reduced weights; agenesis of various regions<br>Testes: increased weight due to edema; small or flaccid<br>Seminal vesicles: malformed<br>Coagulating glands: malformed |
| Fisher et al., 2003. Human Repro 18:1-13. | Testes: Cryptorchidism; similar to testicular dysgenesis syndrome; abnormal Sertoli cell-gonocyte interaction<br>Penis and urethra: hypospadias<br>Infertility |
| Kleymenova et al., 2005. Biology of Reproduction 73:482-490. | Testes: Cytoplasmic changes in Sertoli cells w/abnormal cell-cell contact w/gonocytes<br>Seminiferous tubules: clustering of gonocytes in the middle of tubules; altered morphometry; clusters of interstitial cells; decreased no. tubular cross sections/testicular section; increased no. of multinucleated gonocytes |
| Liu et al., 2005. Biol Reprod. 73:180-92. | Anogenital distance: significant reduction |
| Mahood et al., 2005. Endocrinology 146:613-623. | Testes: Aggregation of fetal Leydig cells; reduced Leydig cell size; decreased testosterone levels at GD 19.5 and 21.5 |
| Mylchreest et al., 2002. Repro Toxicol 16:19-28. | Testes: atrophy; Leydig cell hyperplasia; decreased testicular testosterone<br>Seminiferous tubules: enlarged w/ multinucleated gonocytes<br>Epididymides: fewer ducts |




**DBP Toxicogenomics Studies in Male Reproductive Organs after Developmental Exposure: Dose, Analysis Method, and Target Tissue**

| Study | DBP dose | Toxicogenomic method | Tissue collected |
|---|---|---|---|
| Barlow et al. Toxicol Sci. 2003;73(2):431-41. | 500 mg/kg/day | RT-PCR only | testis |
| Bowman et al. Toxicol Sci. 2005;86(1):161-74. | 500 mg/kg/day | Microarray and RT-PCR | Wolffian ducts |
| Lehmann et al. Toxicol Sci. 2004;81(1):60-8. | 0.1, 1.0, 10, 50, 100, or 500 mg/kg/day | RT-PCR only | testis |
| Liu et al. Biol Reprod. 2005;73(1):180-92. | 500 mg/kg/day | Microarray and RT-PCR | testis |
| Shultz et al.. Toxicol Sci. 2001;64(2):233-42. | 500 mg/kg/day | Microarray and RT-PCR | testis |
| Thompson et al. Endocrinology. 2004;145(3):1227-37. | 500 mg/kg/day | RT-PCR only | testis |
| Thompson et al. Biol Reprod. 2005;73(5):908-17. | 500 mg/kg/day | Microarray and RT-PCR | testis |
| Wilson et al. Toxicol Lett. 2004;146(3):207-15. | 750 or 1000 mg/kg/day | RT-PCR only | testis |

**All studies treated Sprague-Dawley Rats *in utero* with DBP by oral gavage.**

## Future Steps

- **Develop generic approach to utilizing TG data in risk assessment**
- **Internal review draft of the case study report**
- **Agency colloquium to review the case study**

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